RESIDUES AND TRACE ELEMENTS

Use of Buffering and Other Means to Improve Results of Problematic Pesticides in a Fast and Easy Method for Residue Analysis of Fruits and Vegetables

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A modification that entails the use of buffering during extraction was made to further improve results for certain problematic pesticides (e.g., folpet, dichlofluanid, chlorothalonil, and pymetrozine) in a simple, fast, and inexpensive method for the determination of pesticides in produce. The method, known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues in foods, now involves the extraction of the sample with acetonitrile (MeCN) containing 1% acetic acid (HAc) and simultaneous liquid–liquid partitioning formed by adding anhydrous $MgSO_4$ plus sodium acetate (NaAc). The extraction method is carried out by shaking a centrifuge tube which contains 1 mL of 1% HAc in MeCN plus 0.4 g anhydrous $MgSO_4$ and 0.1 g anhydrous NaAc per g sample. The tube is then centrifuged, and a portion of the extract is transferred to a tube containing 50 mg primary secondary amine sorbent plus 150 mg anhydrous $MgSO_4$/mL of extract. After a mixing and centrifugation step, the extract is transferred to autosampler vials for concurrent analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry. Independent of the original sample pH, the use of buffering during the extraction yields pH <4 in the MeCN extract and >5 in the water phase, which increases recoveries of both acid- and base-sensitive pesticides. The method was evaluated for 32 diverse pesticides in different matrixes, and typical percent recoveries were 95 10, even for some problematic pesticides. Optional solvent exchange to toluene prior to GC/MS analysis was also evaluated, showing equally good results with the benefit of lower detection limits, but at the cost of more time, material, labor, and expense.

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Anastassiades et al. (1) recently introduced the so-called quick, easy, cheap, effective, rugged, and safe (QuEChERS) method of pesticide residue analysis. In a follow-up study, Lehotay et al. (2) demonstrated its effectiveness for >200 pesticides in lettuce, orange, and several other matrixes using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for analysis. In this latter study, lower recoveries were obtained for the relatively basic insecticide, pymetrozine, in the acidic orange matrix. Degradation of base-sensitive pesticides, such as captan, folpet, dichlofluanid, and chlorothalonil, was also observed, especially in nonacidic matrixes such as lettuce. Problems in the analysis of these pesticides are well known in the pesticide community and, typically, they are merely screened in multiclass, multiresidue methods and require specialized techniques for more accurate analysis (3–5).

Also, unlike traditional methods, the QuEChERS method does not entail a solvent evaporation step to further concentrate the analytes in the final extract prior to analysis. Instead, the method relies on large volume injection (LVI) in GC analysis if the 4–10 mg sample equivalent common in existing methods must be injected. However, LVI is more complicated and expensive than simple splitless injection in GC, and many laboratories do not have LVI devices coupled to their GC instruments. Furthermore, some LVI devices are better than others, and LVI can be problematic in the analysis of certain relatively volatile and problematic analytes (6–8). Some pesticide chemists also have a concern that acetonitrile (MeCN) is the final extract solvent in the QuEChERS method, which is not ideal for splitless injection in GC. MeCN is better than acetone, hexane, and iso-octane for GC analysis in several respects (9), but not in others, i.e., it has a larger expansion volume during vaporization, interferes with selective detectors that are sensitive to nitrogen, and does not halt the degradation of certain pesticides.

Therefore, we sought to investigate alternative procedures in the QuEChERS sample preparation method to possibly provide analyses with a lower limit of quantitation (LOQ) for laboratories that do not possess LVI devices. Toluene was found to be an excellent all-around solvent for GC analysis of pesticides (9), and because it is also miscible with MeCN, we

propose an option to solvent exchange and concentrate the QuEChERS extract in toluene prior to GC analysis.

The aim of this study was to improve the recoveries of problematic pesticides independent of matrix, without sacrificing recoveries of other pesticides. The effect of pH in extraction was to be evaluated as well as the effect of solvent exchange to toluene and additional solid-phase extraction (SPE) cleanup.

Experimental

Apparatus

(**a**) *GC/MS instrument.*—The extracts were analyzed with a Hewlett-Packard (Agilent, Little Falls, DE) 5890 Series II GC and 5972 MS instrument. Electron ionization (EI) was applied in the MS instrument, which typically was run in selected ion monitoring (SIM) mode to improve sensitivity, but full scan operation was also employed in some experiments to better show the matrix background of extracts. The system was equipped with a split/splitless injection inlet, electronic pressure control, and a 7673A autosampler. Chemstation software was used for instrument control and data analysis.

(**b**) *LC/MS/MS instrument.*—The extracts were also analyzed with an Applied Biosystems (Toronto, Canada) API 3000 triple quadrupole instrument using electrospray ionization (ESI). The LC instrument was an Agilent 1100 with a binary pump that also contained a Model WPALS autosampler, and Analyst software was used for instrument control and data analysis.

(**c**) *Chopper and Vortex mixer.*—A 1 L volume RSI 2Y1 Robot Coupe (Ridgeland, MS) chopper was used to comminute fruit and vegetable samples. A standard laboratory Vortex mixer was used to swirl the tubes when extracts were solvent-exchanged to toluene.

(**d**) *Centrifuges.*—A Sorvall RT6000B centrifuge (Newtown, CT) and a Hill Scientific mv13 (Derby, CT) minicentrifuge were utilized for the 50 and 2 mL centrifuge tubes, respectively.

(**e**) *Liquid dispensers.*—An adjustable-volume solvent dispenser was used to conveniently provide 15 mL aliquots of extraction solvent. An adjustable repeating pipet was used to transfer the 0.25, 0.75, and 1.5 mL volumes to autosampler vials for analysis of the extracts. Adjustable pipets were also used to fortify samples, add the internal standard (IS) solution, and prepare calibration standards.

(**f**) *Analytical balances.*—An Ohaus (Florham Park, NJ) GT480 top-loading balance was used to weigh the chopped samples and solid reagents. A Sartorius (Westbury, NY) R160P microbalance was used in the preparation of stock standard solutions and to weigh the tubes in experiments that determined the amount of matrix coextractives from different sample preparation conditions.

(**g**) *Vials and vessels.*—For the extraction step, 50 mL fluorinated ethylene propylene (FEP) centrifuge tubes (Nalgene, Rochester, NY) were employed. Preweighed salt mixtures were stored in sealed 20 mL glass vials. Either 2 mL minicentrifuge tubes or 15 mL graduated centrifuge tubes were used for dispersive SPE in the method, depending on the extract volume needed. Graduated centrifuge tubes were also used for solvent evaporation and exchange, if needed. Standard 2 mL dark glass autosampler vials were used to contain the final extracts.

(**h**) *Solvent evaporator.*—A Zymark (Hopkinton, MA) Turbovap LV evaporator was employed to concentrate the extracts and permit solvent exchange, when needed. The evaporator was also used to take extracts to dryness so that the amount of coextracted matrix components could be measured in different experiments.

(**i**) *pH meter.*—A Radiometer (Cleveland, OH) Model PHM85 pH meter with standard glass electrode was used in experiments to measure pH at room temperature. The meter was tested using calibration buffers before and after each experiment to verify the accuracy of the readings. MeCN solutions were diluted 4-fold with deionized water prior to making measurements, and calibration experiments demonstrated that the pH value was increased linearly by a factor of 0.01 pH units/1% of MeCN in water (i.e., a 0.25 pH value increase for an aqueous solution of 25% MeCN).

Reagents

(**a**) *MeCN, methanol (MeOH), toluene, and water.*—The organic solvents, from Burdick & Jackson (Muskegon, MI), were of sufficient quality for pesticide residue analysis. Ultrapure water from a Barnstead (Dubuque, IA) water purification system was used for preparing the LC mobile phase and other aqueous solutions.

(**b**) *$MgSO_4$, sodium acetetate (NaAc), and NaCl.*—Certified anhydrous $MgSO_4$, ACS grade anhydrous NaAc, and ACS grade NaCl were obtained from Fisher (Fair Lawn, NJ), ICN Biochemicals (Cleveland, OH), and Mallinckrodt (Paris, KY), respectively. The $MgSO_4$ was baked for 5 h at 500 C in a muffle furnace to remove phthalates and residual water.

(**c**) *Acids, bases, and gases*.—Glacial acetic acid (HAc) and double-distilled formic acid (88% purity) were obtained from Mallinckrodt and GFS Chemicals (Columbus, OH), respectively. Solutions were prepared as needed. For %HAc solutions in MeCN, the % indicates the volume fraction of glacial HAc added to MeCN, typically in a 1 L graduated cylinder (e.g., 1% HAc in MeCN was 990 mL MeCN + 10 mL HAc). HCl and NaOH solutions of 1–2M were also used in experiments to adjust the pH of orange juice samples. Ultrahigh purity He for GC/MS and liquid headspace supplied N_2 for LC/MS/MS and solvent evaporation were obtained from Air Products (Allentown, PA).

(**d**) *Pesticide standards.*—Pesticide reference standards were obtained from the National Pesticide Standard Repository of the U.S. Environmental Protection Agency (Fort Meade, MD), Dr. Ehrenstorfer (Augsburg, Germany), Ultra Scientific (North Kingstown, RI), and Chemservice (West Chester, PA). Stock solutions of 1000–2000 g/mL were prepared in various solvents, and working standard pesticide mixtures of 0.75–50 g/mL (depending on the

experiment) were prepared in MeCN. An ethoprophos solution in MeCN was added to samples and/or standards to serve as the IS in experiments.

(**e**) *SPE sorbents and cartridges.*—Primary secondary amine (PSA) sorbent and 500 mg cartridges (40 m particle size) were obtained from Varian (Harbor City, CA). Graphitized carbon black (GCB) sorbent and 500 mg cartridges were obtained from Supelco (Bellefonte, PA) as ENVI-Carb (120/400 sieved fraction) and 250 mg cartridges from Restek (Bellefonte, PA) as CarboPrep-90 for comparison purposes.

(**f**) *Fruit and vegetable samples.*—Blank samples of cucumber, peach, green pepper, plum, orange, orange juice, Romaine lettuce, and other commodities used in experiments were purchased from a local organic produce store. The pits were removed from stone fruits, but peels were not removed from oranges. These samples were well homogenized in the chopper, placed in plastic storage bags, and stored at –40 C until they were needed in fortification experiments and as matrix blanks for matrix-matched calibration standards.

Extraction and Cleanup Procedure

The streamlined procedure given below was used for extraction and cleanup in the final method. Any alterations made in the method in experiments will be mentioned when discussing the results. (*1*) Weigh 15.00 0.05 g of thoroughly comminuted sample into a 50 mL FEP centrifuge tube (use 13 mL water as a reagent blank). (*2*) If needed, fortify the sample with the appropriate volume of spiking solution. (*3*) Add 15 mL 1% HAc in MeCN (v/v) extraction solvent into each tube using the solvent dispenser and ethoprophos IS solution in MeCN to all samples except blanks to yield a 100 ng/g concentration. (*4*) Add 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc (do not get the powders in the threads or rims of the tubes). (*5*) Seal the tubes and shake vigorously for 1 min by hand (3–5 tubes can be held per hand) ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently. (*6*) Centrifuge the tubes at 3450 rcf (5000 rpm on the centrifuge we used) for 1 min. (*7*) Transfer the extracts (upper layer) to dispersive-SPE tubes containing 50 mg PSA + 150 mg anhydrous $MgSO_4$/mL of extract. (*8*) Cap the tubes well and mix the extract with the sorbent/dessicant for 20 s. (*9*) Repeat step 6.

Optional Concentration Step and Solvent Exchange to Toluene

The extract obtained after step 9 is ready for GC/MS analysis directly and LC/MS/MS analysis after 4-fold dilution with aqueous formic acid solution. An LOQ <10 ng/g can be achieved with this MeCN extract for many pesticides analyzed using typical analytical techniques (depending on the amount injected, chromatographic separation, MS technique, instrument sensitivity, and extent of matrix background). If matrix is not the limiting source of noise, the LOQ may be further reduced in GC analysis by concentrating the final extract and exchanging the solvent to toluene. In this case, the procedure continues as follows: (*10*) Transfer 5 mL of the extract to a 15 mL graduated centrifuge tube, and add 1 mL toluene. (*11*) Evaporate the extract to 0.3–0.5 mL using the Turbovap set at 50 C and 7.5 psi N_2 flow. (*12*) Bring the extracts to 1.0 mL with toluene, then add 150 mg anhydrous $MgSO_4$ to remove any residual water. (*13*) Vortex the tubes to rinse the walls above the 6 mL mark. (*14*) Centrifuge the tubes as in step 6 above. This extract may still contain MeCN, thus precautions should be taken (e.g., solvent venting and/or a more complete solvent exchange) prior to conducting GC analysis using a nitrogen-phosphorus detector (NPD) or other detector adversely affected by N-containing solvent.

Preparation of Matrix-Matched Calibration Standards

For analyses, the extracts from step 9 for both GC/MS and LC/MS/MS (or toluene extracts from step 14 for GC/MS only) were transferred to labeled autosampler vials. For calibration in fortification experiments, matrix-matched standards were prepared by adding the appropriate volumes of the pesticide spiking mixture and IS solutions to blank extracts. When solvent exchange to toluene was performed, this was done before bringing the evaporated extract to the 1 mL mark with toluene. In the case of MeCN extracts for GC/MS, an appropriate volume of MeCN was added to all vials to give consistent total volumes prior to the transfer of 0.25 mL to a second autosampler vial for LC/MS/MS analysis. After this transfer, 0.75 mL 6.67mM formic acid solution in water was added, and all vials were capped and shaken before they were placed in the autosampler trays for concurrent analyses by GC/MS and LC/MS/MS.

GC/MS and LC/MS/MS Analyses

The GC separation was conducted on a Restek Rtx-5ms capillary column (30 m, 0.25 mm id, 0.25 m film thickness) with the following conditions: He constant flow, 1 mL/min; inlet temperature, 250 C; injection volume, 1 L (splitless); MS transfer line temperature, 290 C; initial oven temperature, 95 C, held for 1.5 min, then a 20 C/min ramp to 180 C followed by a 5 C/min ramp to 230 C and a 25 C/min ramp to 290 (held for 10 min). The quadrupole was operated in SIM mode with EI, and the multiplier was set 200 V above the autotuned setting. For background analyses of matrix coextractives, full scan (50–350 *m/z*) was employed. Table 1 gives the particular retention times and quantitation ions for the SIM mode analysis of the pesticides.

In the case of LC/MS/MS, the analytical column was a 150 mm 3 mm id, 3 m particle size LUNA C_{18}-2 obtained from Phenomenex (Torrance, CA). A 4 mm 3 mm id guard column of the same stationary phase was also used. The injection volume was 10 L. A gradient elution program at 0.3 mL/min flow, in which both reservoirs contained 5mM formic acid in (A) water and (B) MeOH, was used as follows: 25% solution B ramped to 100% linearly over 15 min then held for an additional 15 min. After 30 min, the flow was increased to 0.5 mL/min, and the mobile phase was returned to 75% solution A over the course of 2 min and allowed to

Table 1. Conditions for the GC/MS (SIM) analysis of representative pesticides and degradation products included in this study

Analyte	t_R[a], min	Quantitation ion, *m/z*
Methamidophos	5.95	141
Dichlorvos	6.02	185
Acephate	7.44	136
Propoxur	8.96	152
Ethoprophos (IS)	9.22	200
Hexachlorobenzene	10.15	284
Lindane	10.79	219
Diazinon	10.89	304
Chlorothalonil	11.26	266
Chlorpyrifos-methyl	12.17	286
Carbaryl	12.49	144
Dichlofluanid	13.20	224
Chlorpyrifos	13.44	314
Dichlorobenzophenone	13.85	250
Cyprodinil	14.39	224
Penconazole	14.57	248
Tolylfluanid	14.68	238
Heptachlor epoxide	14.80	353
Thiabendazole	14.95	201
Captan	14.98	79
Folpet	15.17	260
cis-Chlordane	15.80	375
Imazalil	16.19	215
p,p'-DDE	16.47	318
Dieldrin	16.63	263
Endosulfan sulfate	18.27	387
Dicofol	19.24	251
cis-Permethrin	20.54	183
trans-Permethrin	20.66	183
Coumaphos	20.73	362

[a] t_R = Retention time.

equilibrate for 6 min. A Valco (Houston, TX) divert valve was placed between the column outlet and ESI source to eliminate the introduction of salts and other early eluting matrix components from the extracts into the MS instrument at the beginning of the chromatogram (<8 min) and any coextracted matrix components eluting >23 min.

The ESI source was used in the positive mode, and N_2 nebulizer, curtain, and other gas settings were optimized according to recommendations made by the manufacturer; source temperature was 550 C, ion spray potential, 4500 V, decluster potential (or cone voltage), 30 V, focus potential, 131 V, and entrance potential, 10 V. A Harvard Apparatus (Holliston, MA) syringe pump was used to introduce individual pesticide solutions into the MS instrument to allow optimization of the MS/MS conditions, which are shown in Table 2. All 15 analyte transitions from 8–23 min with 50 ms dwell times were monitored in a single segment.

Results and Discussion

During the development of the original QuEChERS method (1), it was noted that variable results were obtained for captan and chlorothalonil. In follow-up validation experiments (2), dicofol, folpet, dichlofluanid, tolylfluanid, and certain other pesticides were also found to be problematic in the QuEChERS method. Figure 1 gives the structures of problematic analytes of interest. These pesticides are notoriously difficult, and it is not unusual for existing methods to sacrifice performance of them in multiclass, multiresidue monitoring methods in order to attain better overall performance for a large number of other residues in an efficient manner. In Figure 1, note that folpet, captan, dichlofluanid, and tolylfluanid share a similar *N*-trihalomethylthio functional group, which is the source of their instability. At basic pH, in certain solvent and matrix environments, and/or at elevated temperatures, captan (and captafol) degrade to tetrahydrophthalimide, folpet converts to phthalimide, and dichlofluanid and tolylfluanid become *N,N*-dimethyl-*N*-phenylsulphamide (DMSA) and dimethylamino-sulfotoluidide (DMST), respectively. Furthermore, dicofol readily converts to dichlorobenzophenone, and chlorothalonil degrades to 4-hydroxy-2,5,6-trichloroisophthalonitrile. These degradation products are not part of the residue definition for enforcement applications (10), but they are still often monitored in GC/MS methods to assess the original presence of the parent pesticides. In cases when high metabolite concentrations are found, separate analysis can be conducted to provide more accurate results using specifically targeted methods of sample preparation and/or analysis (3–5).

The causes of the problems for these analytes are diverse and hard to control. For example, parameters that can factor into the rate of degradation include pH, type of solvent (including lot-to-lot variability), light intensity, matrix components and their concentrations, temperature, water content, and analyte concentration. These pesticides are also susceptible to adverse effects in the GC injection port, column, and MS ion source. Their EI mass spectra do not generally exhibit the molecular ion, with the detection of captan being especially difficult due to its dissociation to weak ions of relatively low mass in MS. Moreover, except for dichlofluanid and tolylfluanid, they do not work well in atmospheric pressure ionization techniques in LC/MS in either the positive or negative ion modes (2). Thus, when poor results are obtained for these analytes, it is usually very difficult to assess whether analyte degradation, sample processing, extraction, cleanup, injection, separation, and/or detection are the cause(s) of the poor results.

Table 2. Conditions for the LC/MS/MS analysis of representative pesticides included in this study

Analyte	t_R[a], min	Precursor ion, *m/z*	Product ion, *m/z*	Collision energy, V
Methamidophos	9.6	141.8	112.0	17
Pymetrozine	10.02[b]	217.9	105.0	27
Acephate	11.1	183.8	143.0	19
Carbendazim	11.9	191.8	160.0	25
Thiabendazole	13.2	201.8	174.9	37
Imidacloprid	15.9	255.9	209.0	21
Imazalil	16.3	296.8	159.0	31
Thiophanate-methyl	18.8	342.8	151.0	29
Dichlorvos	19.0	220.7	127.0	23
Carbaryl	19.3	202.2	145.0	13
Dichlofluanid	20.9	332.7	223.8	17
Ethoprophos (IS)	21.2	242.8	173.0	21
Cyprodinil	21.2	225.9	108.0	35
Tolylfluanid	21.3	346.7	237.9	15
Penconazole	21.5	283.8	159.0	39

[a] t_R = Retention time.
[b] First of 2 peaks.

Maintaining Acidic Conditions

Except for pymetrozine, the problematic pesticides shown in Figure 1 are all base-sensitive (i.e., they degrade more readily as pH increases). Few common pesticides in multiresidue monitoring applications degrade at acidic conditions. In previous experiments (9), we found that adding 0.1% HAc (v/v) to MeCN solutions helped prolong the stability of the problematic pesticides prior to analysis. The obvious possible solution to degradation problems during sample preparation is to maintain low pH throughout the procedure. In an experiment to assess this possibility, HAc was added at different amounts (0.1, 0.5, and 1%) to MeCN extraction solvent, and the recoveries from the original method for a number of pesticides were determined at 500 ng/g fortified in a mixed commodity matrix.

Figure 2 provides the GC/MS results from this experiment for 11 pesticides of particular interest normalized to the IS (the other fortified pesticides gave consistent results independent of HAc content). Surprisingly, clear improvements in recoveries were not observed for those pesticides as HAc content increased. As the results indicate, chlorothalonil and dichlofluanid gave substantially lower recoveries (<30%)

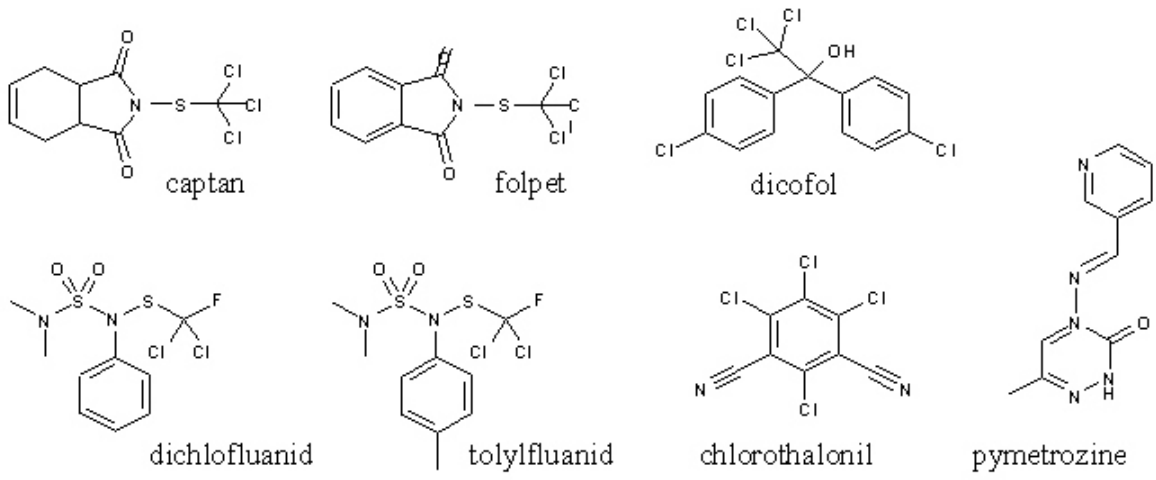

Figure 1. Chemical structures of 7 problematic pesticides for multiclass, multiresidue analysis.

than the other pesticides in this experiment, and a strong effect was not observed versus HAc content. Dichlofluanid is the most sensitive *N*-trihalomethylthio fungicide in terms of degradation in MeCN (9) and serves as a good indicator for the others in that group. Only dicofol appears to have been recovered better using HAc than without it in MeCN (this was also demonstrated by the increased "recovery" of its degradation product, dichlorobenzophenone, which was 230% when using MeCN alone and averaged 140% when HAc was added). The main conclusion from this experiment was that the addition of HAc to MeCN extraction solvent (and continuing to use NaCl + $MgSO_4$ for the partitioning step) did not provide significant benefits to warrant changing the original method in this manner.

Control of pH

In the original QuEChERS study, the most basic pesticides evaluated were thiabendazole and imazalil. Experiments showed no effect on the recovery of these basic pesticides from pH 2–7 in apple juice with the QuEChERS method, but when ethyl acetate was used for extraction, lower pH was found to reduce their recoveries (1). However, in a follow-up study of the QuEChERS method involving 229 pesticides in oranges and lettuce (2), pH of the sample was found to have an effect on recoveries of several pesticides (e.g., asulam, florasulam, cycloxydim, sethoxydim, ethirimol, and thiophanate-methyl). Pymetrozine was the most notable example, which gave a lower recovery (ca 25%) in the acidic orange matrix, whereas recovery was ca 80% in the less acidic lettuce (2). Because pymetrozine is also registered for application on citrus fruits (10), its recoveries should be improved for regulatory purposes. Thus, pymetrozine was chosen as a good indicating analyte in this study. Figure 1 provides the structure of pymetrozine at neutral conditions, but it either hydrolyzes as pH becomes too acidic (11), or becomes cationic and does not partition well into the MeCN phase.

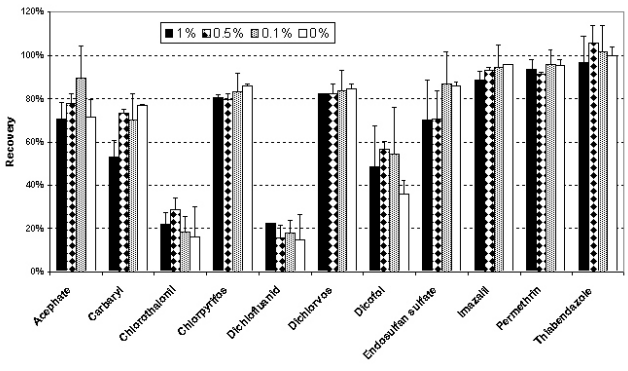

Figure 2. Recoveries of representative pesticides fortified at 500 ng/g in a mixture of peach, plum, cucumber, and green pepper (1 part each by weight) extracted using the original QuEChERS method (0% HAc) or using 1, 0.5, or 0.1% HAc in MeCN (v/v) as the extraction solvent. Error bars represent the standard deviation (*n* = 2).

Therefore, not only was pH an important parameter in the stability of several base-sensitive pesticides, it was also critical for acid-sensitive pesticides such as pymetrozine. However, a pH of 6–7 is ideal for the recovery of pymetrozine, whereas pH <4 would be better for other pesticides listed in Figure 1. This situation called for a compromise in which pH of 4–5 would be maintained to give adequately high recoveries of pymetrozine (>70%) and sufficient stability of the base-sensitive pesticides for their analysis. Buffering of the extracts was a reasonable approach to pursue in experiments, and the most appropriate buffer for the 4–5 pH range was HAc ($pK_a = 4.75$) with an acetate salt. HAc is rather inexpensive, safe, and readily available, and NaAc was chosen because it is the typical buffering salt for HAc. HAc and NaAc are already naturally present in many fruits, thus new potential analytical interferences or undesired effects would be less likely to occur.

Furthermore, we did not wish to create additional steps in the QuEChERS method, and the simplest modification to achieve a buffering effect was to add HAc to the MeCN extraction solvent and replace NaCl with NaAc in the salting out step. In actuality, the QuEChERS method became more streamlined than before by using the buffering procedure, because we wanted to induce the buffering effect as soon as possible during extraction. In effect, the 2-step extraction and partitioning procedure in the original method became a single-step procedure. A further study with incurred residues in a variety of commodities should be done to confirm that the separate extraction and partition steps are not necessary. We should note, however, that even in the original method, some sample types, such as apples, generated 2 phases during the initial extraction step with MeCN due to the high sugar content, and high pesticide recoveries were still achieved (1).

Initial experiments were designed to determine the amount(s) of HAc and NaAc that should be used to control pH of various fruit and vegetable matrixes. Figure 3 shows the effect of adding increasing amounts of NaAc (along with constant amount of $MgSO_4$) to orange juice extracted with 1% HAc in MeCN. The pH values were recorded in both the bottom water layer and the upper MeCN extract (after a 4-fold dilution with water) and, as the figure shows, an increasing amount of NaAc up to 1.5 g/15 g of sample caused the acidic orange juice to become more basic. An unexpected effect was observed, in that the MeCN extract was ca 1.5 pH units more acidic than the water phase in the buffered extracts. As explained in the *Experimental* section, the pH measurement of MeCN solutions was found to be quite accurate, thus the presence of organic solvent was not the cause of this observed pH difference.

We believe that the use of buffering leads to greater partitioning of the HAc (and probably other organic acids in the sample) to the MeCN extract, and acetate salt predominantly remains in the water phase. We had anticipated that pH would be the same (ca 5) in both phases and were surprised by this effect. In any case, the effect was thought to

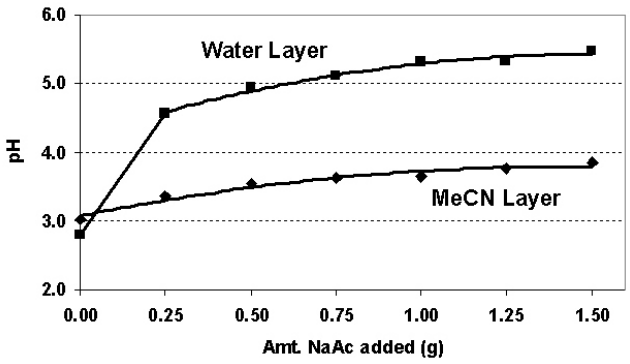

Figure 3. Effect on pH of the 2 layers formed during extraction of 15 g orange juice with 15 mL 1% HAc in MeCN followed by the addition of 6 g anhydrous $MgSO_4$ + varying amount of anhydrous NaAc.

be beneficial in the pesticide extraction process because the base-sensitive pesticides in Figure 1 would preferentially partition into the MeCN, where they would be better protected by the lower pH (or stabilizing coextractives), and the higher pH of the water phase would better neutralize or stabilize the basic pesticides to increase their partitioning into the organic phase.

Another experiment was designed to show the buffering effect of the HAc/NaAc procedure with respect to sample pH by adding HCl or NaOH solutions to orange juice until the pH was 2.00, 3.25, 4.50, 5.75, and 7.00, which covers the 2–7 pH range of fruits and vegetables. Figure 4A demonstrates how buffering stabilized the pH of the water phase to 5.6–6.0 in the extraction step but, without buffering (the original QuEChERS method), pH increased from 2.5 to 6.8, which correlated with sample pH from 2–7. As shown in Figure 4A and B, the pH of both the MeCN and water layers more or less tracked together relative to pH of the sample when using the original QuEChERS method (1 mL MeCN + 0.1 g NaCl + 0.4 g $MgSO_4$/g sample). In other experiments using this procedure, the pH of the water layer was 2.8 for tomato, peach, and apple extracts; 3.0 for grape; and 4.3 for green pepper. However, the buffered QuEChERS method (1 mL 1% HAc in MeCN + 0.1 g NaAc + 0.4 g $MgSO_4$/g sample) gave a similar effect as shown in Figure 3, in that the pH of the initial MeCN extract was ca 2 pH units more acidic (pH ca 3.7) than the water phase (pH ca 5.7), independent of original pH of the sample. Clearly, the buffering procedure provided the desired effect to increase the pH of highly acidic commodities and reduce pH of less acidic ones during extraction. This situation was believed to be ideal for improved extraction of the problematic pesticides.

Another critical aspect shown in Figure 4B pertains to the effect of the buffering procedure on the dispersive-SPE cleanup step with PSA sorbent + $MgSO_4$. PSA was observed to remove acidic components from the extract as discussed previously (1), but the extent of this effect on pH was not measured before. As the plot shows, the PSA cleanup step in the original method decreased acidity of the extract by 2–3 pH units. As already discussed, acidic conditions promote stability and recovery of certain base-sensitive pesticides, and this result helps explain the losses of the base-sensitive pesticides shown in Figure 1, and may also explain the lower recoveries of certain pesticides shown in Figure 2, despite the use of an acidified extraction solvent. On the other hand, pH essentially remained the same (ca 3.5–3.7) before and after cleanup in the buffered method, which was a very beneficial situation.

Effect of pH and Buffering on Coextractives

The modification to use buffering in the QuEChERS method had stabilized pH, but how much buffering strength should be used and what would be its effect on cleanup? In the experiment described above, the amounts of coextractives from the orange juice samples were determined by weight difference after evaporation of the extracts to dryness in

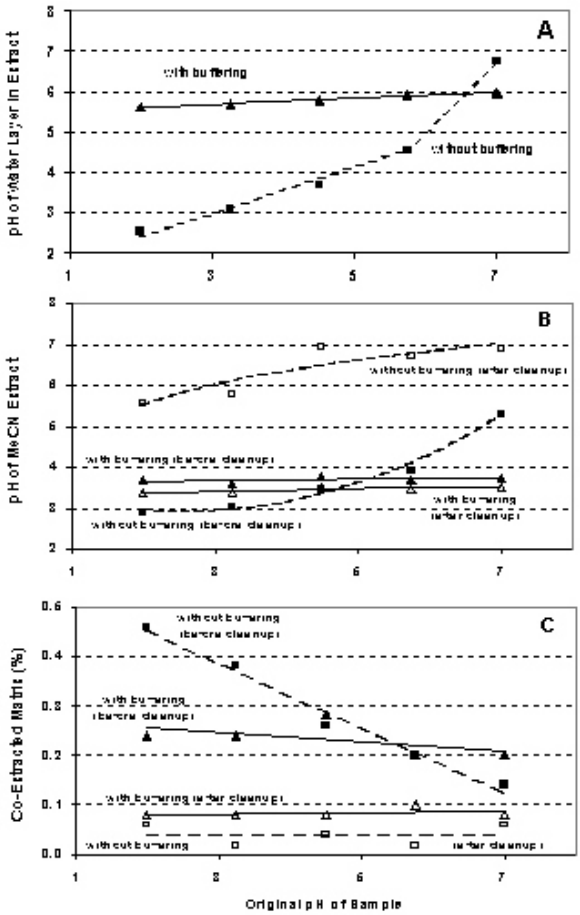

Figure 4. Effect of buffering (use of 1% HAc in MeCN + 0.1 g anhydrous NaAc + 0.4 g $MgSO_4$/g sample) during extraction of orange juice adjusted to pH 2–7 prior to extraction in comparison with the original QuEChERS procedure: (A) pH of the water layer; (B) pH of the MeCN extracts; and (C) amount of coextractives before and after dispersive-SPE cleanup with PSA sorbent.

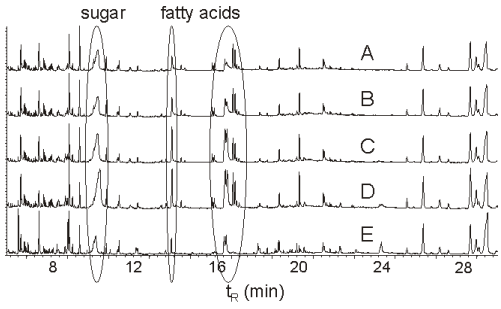

Figure 5. GC/MS full scan total ion chromatograms of orange extracts after dispersive-SPE cleanup with 25 mg PSA + 150 mg anhydrous $MgSO_4$/mL extract in which 15 g samples were extracted with 15 mL solvent and 6 g $MgSO_4$ as well as: (A) 1% HAc in MeCN + 1.5 g NaAc; (B) 0.67% HAc in MeCN + 1 g NaAc; (C) 0.33% HAc in MeCN + 0.5 g NaAc; (D) MeCN and $MgSO_4$ only; and (E) MeCN + 1.5 g NaCl (sample E came from a different batch of oranges than the others).

preweighed test tubes. Similarly, the effect of cleanup was measured after conducting dispersive SPE using 50 mg PSA + 150 mg $MgSO_4$/mL of MeCN extract.

Figure 4C shows the percentage of coextractives in the MeCN extracts plotted with respect to pH of the orange juice samples. Without buffering during extraction, the amount of coextractives from the original sample more than tripled from 0.14 to 0.46% in linear fashion as sample pH decreased from 7 to 2. Conversely, the buffering procedure led to a rather consistent amount of coextractives (0.20–0.28%) from the same samples. After dispersive-SPE cleanup, a large amount of coextractives was removed in all extracts. Without buffering, only 0.02–0.06% of the original mass of the sample remained in the final extract, whereas 0.08–0.10% remained when employing the buffering protocol. This indicated that the relatively high HAc concentration in the extracts slightly reduced the ability of the PSA sorbent to retain bulk matrix coextractives from orange juice, but, still, 50–71% of the coextractives were eliminated by the very simple cleanup step. In terms of amounts, a 5 L injection of the 1 g/mL final MeCN extract (5 mg sample equivalent) contains 4–5 g orange juice matrix components using the modified method versus 1–3 g with the original method. We did not study the factor of long-term ruggedness of the GC system performance in these experiments, but we believe that this potential concern in decreased ruggedness of the method will be overcome in GC by the use of analyte protectants (1, 12, 13) and/or direct sample introduction (7, 8) in the future.

Although bulk coextractives could pose problems related to ruggedness of an analytical method, they do not necessarily interfere in the analysis. To measure possible interferences in the modified QuEChERS method, we analyzed orange extracts (a difficult matrix) using different extraction conditions in full-scan GC/MS. As Figure 5 shows, many peaks from coextracted matrix components appeared at all tested extraction conditions as described in the figure captions. Interestingly, the use of more HAc and NaAc to buffer the extraction decreased the amount of sugar and fatty acid components in the final extracts, as shown in the circled regions of the chromatograms in Figure 5. For comparison purposes, the bottom trace E shows the chromatogram from a different lot of oranges extracted by the original QuEChERS method. The same patterns occurred in this example, but the oranges had somewhat different sugar and fatty acid compositions, making quantitative comparisons difficult. Due to the continued presence of the sugars and fatty acids in the extracts (and other experiences with high sugar fruits), we doubled the amount of PSA used for dispersive-SPE cleanup in the final method from 25 to 50 mg/mL of extract.

Effect of pH and Buffering on Recoveries

The working hypothesis after these experiments (and others not presented) was to employ the highest tested amount of HAc (1%) in MeCN in combination with the same amount of NaAc in the buffered QuEChERS approach as NaCl in the original method (0.1 g/g sample). Perhaps higher buffer concentrations would have provided even cleaner extracts or other interesting results, but we were satisfied with the results thus far with the stated conditions. We were concerned that NaAc would adversely affect the recoveries of certain pesticides in comparison to NaCl, as was shown for LiCl, $NaNO_3$, and other salts during development of the original QuEChERS method (1). Also, our experience was that salts used in conjunction with $MgSO_4$ tended to decrease pesticide recoveries.

Figure 6 provides the recoveries for pesticides of special interest from orange juice samples adjusted to pH 2–7 in a repeat of the experiment described above. The 16 other pesticides detected in the spiking mixture gave excellent and reproducible recoveries without trends versus pH in the experiment in both the original and modified methods (giving similar results as shown for carbaryl, imazalil, and propoxur in the figure). A bias in the ethoprophos IS concentrations occurred in this experiment due to too small (5–15 L) pipetting volumes added to calibration standards (which was corrected in experiments to follow), so the recoveries shown in Figure 6 were normalized to penconazole and lindane in LC/MS/MS and GC/MS, respectively.

As shown in Figure 6A, acephate, carbaryl, carbendazim, imazalil, methamidophos, propoxur, and thiabendazole (typically problematic pesticides in multiresidue methods) exhibited small or no trends in their recoveries versus pH, which agreed with previous experiments involving the original QuEChERS method (1). Acephate gave slightly decreasing recovery versus increasing pH in the nonbuffered method, whereas methamidophos, dichlorobenzophenone, and thiabendazole had the opposite trend. Dichloro-benzophenone is a degradation product of dicofol and, unfortunately, GC conditions were such that dicofol could not be detected in this experiment. In the case of thiophanate-methyl in the nonbuffered method, the

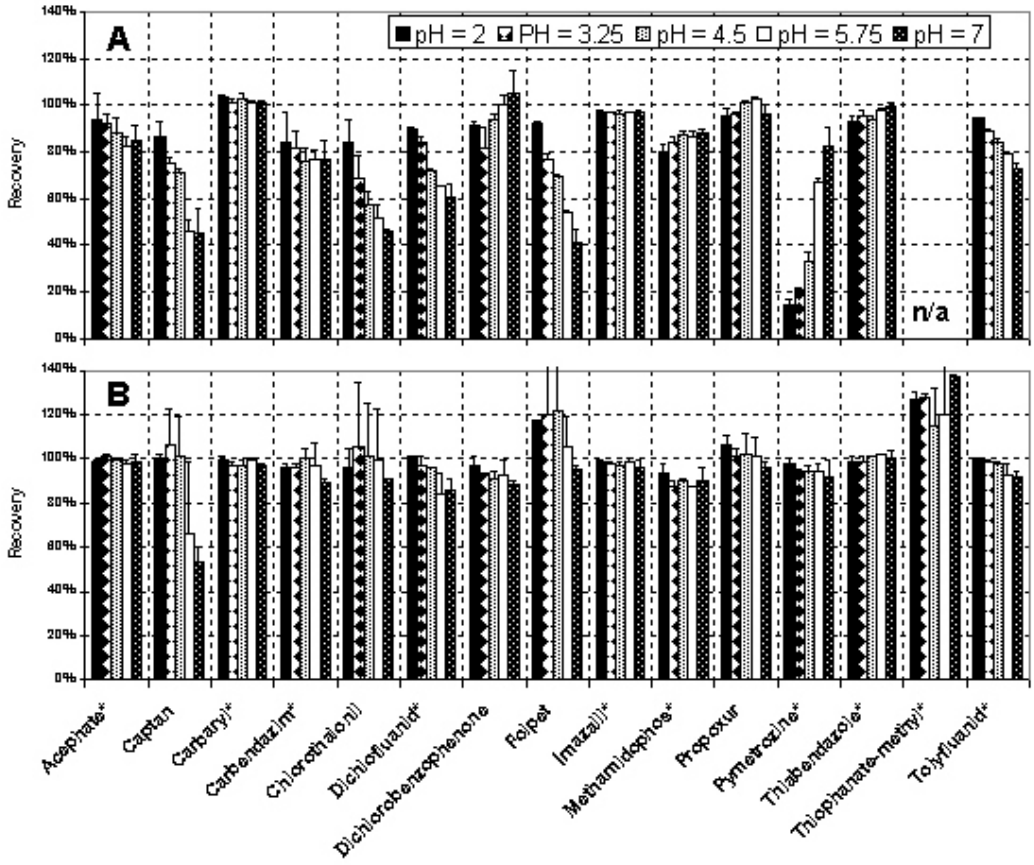

Figure 6. Recoveries of representative pesticides fortified at 500 ng/g in orange juice adjusted to pH 2–7 and extracted using: (A) the original QuEChERS method (1); or (B) the modified method using 1% HAc in MeCN for extraction and NaAc instead of NaCl in combination with $MgSO_4$ for salting out. Error bars represent the standard deviation (n = 2), and the asterisks denote an LC/MS/MS result (otherwise, GC/MS results are presented). The thiophanate-methyl quantitation in nonbuffered conditions was not reliable due to its degradation in both standards and extracts.

quantitation was not possible because the analyte degraded irreproducibly in both the matrix-matched calibration standards and sample extracts. The 20% high bias for thiophanate-methyl in the case of the buffered extracts was also probably due to slight (but reproducible) degradation of the analyte in the calibration standards.

Base-sensitive pesticides (captan, chlorothalonil, dichlofluanid, folpet, and tolylfluanid), degraded versus increasing pH as shown in Figure 6A, which is in agreement with previous results from the original QuEChERS method in orange and lettuce extracts (2). Similarly, pymetrozine results also followed the previously observed trend (2), and its recoveries increased from 15 to 82% with respect to sample pH of 2 to 7.

In each set of circumstances for the different types of pesticides (except possibly in the case of captan), the use of the buffered QuEChERS protocol stabilized these trends. As shown in Figure 6B, the small trends with respect to pH disappeared for acephate, carbendazim, dichlorobenzophenone (representing dicofol), methamidophos, and thiabendazole, and only a minor or no trend was observed for pesticides listed in Figure 6A that had large effects due to pH. Pymetrozine, dichlofluanid, and tolylfluanid recoveries from the modified method were all 80–100%, independent of the original sample pH. Although chlorothalonil and folpet results were more variable than those shown in Figure 6A (due to worsening conditions of the GC system as more samples were being injected), the average recoveries were higher and more consistent overall. Captan was the only pesticide to exhibit a similar trend in both Figure 6A and B, but dichlofluanid was previously shown to be a better indicator of degradation of *N*-trihalomethylthio pesticides (9). Dichlofluanid gave acceptable results in the experiment, thus the captan results are probably due to GC/MS maintenance issues rather than its actual degradation in the extracts (in fact, dichlofluanid and tolylfluanid exhibited the same trend in GC/MS as captan, but not in LC/MS/MS).

Based on the results of this experiment, we concluded that the modifications in the method provided a good possibility of meeting our goal to simultaneously improve recoveries of

both acid-sensitive pesticides (e.g., pymetrozine) and the problematic base-sensitive pesticides (e.g., dichlofluanid and chlorothalonil) while not affecting the recoveries of other pesticides commonly monitored in multiclass, multiresidue methods. Our initial concerns about the use of a rather high acid concentration in the MeCN extraction solvent and replacing NaCl with NaAc in the method were shown to be unfounded. In fact, higher and more consistent recoveries were achieved using HAc/NaAc for a critical pair of pesticides (acephate and methamidophos) that serve as a good quality test for any multiresidue method.

Method Validation

Based on the results from experiments described above, we decided to perform a validation study similar to the one conducted previously for the original method in fortified lettuce and orange (2). To save a great deal of data processing time, we pared the comprehensive list of 229 fortified pesticides from before to 32 carefully chosen ones as representatives of the many different classes and properties. Experience tells us that if the method works well for the selected representative pesticides, then it should work equally well for nearly all of the others monitored routinely in multiclass, multiresidue methods. Essentially as before, 6 replicates, each at 3 spiking levels (10, 50, and 250 ng/g), in Romaine lettuce and orange matrixes were prepared using the buffered QuEChERS method and analyzed by GC/MS and LC/MS/MS.

Table 3 provides the validation results from this experiment for MeCN extracts from the modified method. The GC/MS method was still unable to detect dicofol in this experiment, which is not unusual in practice, thus it was not listed in the table. Dichlorobenzophenone results for both matrixes were excellent (ca 100%) in the validation experiment, which led us to believe that dicofol degradation was not a serious problem during sample preparation, but complete conversion of dicofol to dichlorobenzophenone in the standards and/or gas chromatograph inlet would give the same result. The GC instrument liner and capillary column were also unable to adequately permit sensitive analysis of captan and folpet in most of the extracts, but the 250 ng/g spiked orange samples for those particularly problematic pesticides were able to be analyzed, showing ca 90% recoveries. Dichlofluanid and tolylfluanid were still determined in all extracts by both instruments in the experiment to serve as representives for the other *N*-trihalomethylthio fungicides. The LC/MS/MS results are distinguished from the GC/MS results by superscript b after the name of the pesticide.

As the table shows, average recoveries, repeatabilities, and reproducibilities versus ethoprophos IS were very good in the experiment for nearly all tested pesticides, even at the 10 ng/g level. In oranges, all recoveries were between 60–110%, and only a few isolated instances of <80% recovery and/or >15% relative standard deviation (RSD) occurred. A few instances of high bias in the results for lettuce occurred (quantitation of chlorothalonil, endosulfan sulfate, heptachlor epoxide, and methamidophos were borderline at 10 ng/g), but, again, overall results were excellent.

Pymetrozine gave consistently ca 80% recovery in orange and 91–108% recoveries in lettuce. This is a great improvement in the pymetrozine recoveries obtained from the original QuEChERS method, especially in oranges (a commodity on which the pesticide is applied). Chlorothalonil in all extracts yielded quite variable results, which could not be traced to sample preparation or GC analysis as being the cause of the problem. Ultimately, it was probably a combination of both factors.

As in the previous study (2), LC/MS/MS generally provided more reliable results than GC/MS. Thus, curious GC/MS results, such as the 135 23% recovery at 250 ng/g for carbaryl in lettuce or its 69 4% recovery at 10 ng/g in orange, can be negated by the more reasonable LC/MS/MS results (e.g., 100 2 and 94 3% recovery for carbaryl in the same cases, respectively). Interestingly, this periodic high bias in carbamate results by GC/MS was also observed previously (2). The other pesticides detected on both instruments did not give discrepancies in the results except for dichlofluanid and tolylfluanid in lettuce. The LC/MS/MS results showed 78 11% recovery overall for both pesticides in lettuce, but GC/MS gave 98 13 and 102 16% recoveries for dichlofluanid and tolylfluanid, respectively. Both analyses provided similar recoveries for the base-sensitive pesticides in oranges (81–82% recoveries of dichlofluanid and 87–96% for tolylfluanid), thus we feel that the LC/MS/MS results for lettuce also make more sense. Unfortunately, LC/MS/MS was not able to resolve the nonexistent or less trustworthy GC/MS results for chlorothalonil, dicofol, captan, and folpet.

The buffered QuEChERS sample preparation method still gave acceptable recoveries and RSDs for the tested pesticides for regulatory monitoring purposes except, perhaps, for chlorothalonil. Chlorothalonil is especially problematic in any case, and typically requires special precautions during sample homogenization, preparation, and analysis (14). The GC/MS method was the source of problems for direct analysis of dicofol, folpet, and captan (and probably chlorothalonil), and we recommend that their degradation products continue to be monitored as is customary currently in most pesticide analysis protocols.

Solvent Exchange to Toluene

One of the potential limitations of the QuEChERS method is the relatively dilute 1 g/mL final extract concentration in MeCN for GC/MS analysis. Traditional methods typically involve time-consuming solvent exchange and extract concentration steps prior to GC analysis to yield final extract concentrations of 2–5 g/mL in a nonpolar organic solvent. We recently demonstrated that MeCN with 0.1% HAc provided acceptable pesticide stability and GC injection characteristics for multiresidue pesticide analysis (9). Also, Table 3 demonstrates how an LOQ of 10 ng/g can still be achieved for many common pesticides by standard GC/MS (SIM mode) for a 1 mg sample equivalent injection. Regulatory tolerances of pesticides are typically 50–10 000 ng/g (10), and lower LOQ

Table 3. Average % recoveries (%RSD) of fortified pesticides in lettuce and orange from the buffered QuEChERS method with GC/MS and LC/MS/MS analyses (MeCN final extracts)[a]

	Romaine lettuce				Orange				
Pesticide	10 ng/g (*n* = 6)	50 ng/g (*n* = 6)	250 ng/g (*n* = 6)	Overall (*n* = 18)	10 ng/g (*n* = 6)	50 ng/g (*n* = 6)	250 ng/g (*n* = 6)	Overall (*n* = 18)	Overall (*n* = 36)
Acephate[b]	105 (12)	97 (4)	91 (2)	98 (10)	90 (2)	85 (2)	86 (2)	87 (6)	93 (10)
Captan	—-	—-	—-	—-	—-	—-	93 (11)	—-	—-
Carbaryl	105 (7)	89 (6)	**135** (**17**)	110 (**21**)	69 (6)	94 (11)	103 (11)	88 (**19**)	99 (**23**)
Carbaryl[b]	104 (5)	99 (3)	100 (2)	101 (4)	94 (3)	93 (3)	96 (3)	95 (3)	98 (5)
Carbendazim[b]	105 (8)	104 (2)	107 (2)	105 (5)	88 (2)	88 (2)	91 (2)	89 (3)	97 (9)
Chlordane	104 (4)	97 (3)	99 (6)	100 (5)	92 (4)	99 (4)	97 (6)	96 (5)	98 (6)
Chlorothalonil	**135** (11)	103 (11)	**118** (**16**)	**119** (**16**)	—-	61 (10)	84 (**17**)	72 (**21**)	100 (**29**)
Chlorpyrifos	99 (6)	101 (3)	100 (6)	100 (5)	97 (4)	100 (6)	99 (4)	99 (4)	99 (5)
Chlorpyrifos-methyl	105 (3)	99 (2)	101 (5)	101 (4)	97 (6)	101 (3)	100 (4)	99 (4)	100 (4)
Coumaphos	106 (9)	99 (7)	95 (7)	100 (8)	98 (9)	104 (11)	104 (8)	102 (9)	101 (9)
Cyprodinil	107 (2)	99 (3)	100 (6)	102 (5)	90 (4)	96 (5)	96 (5)	94 (5)	98 (6)
Cyprodinil[b]	101 (3)	97 (1)	100 (2)	100 (3)	98 (3)	95 (3)	97 (3)	97 (3)	98 (3)
DDE	103 (4)	96 (3)	100 (6)	99 (5)	92 (2)	96 (6)	97 (6)	95 (5)	97 (6)
Diazinon	101 (5)	99 (2)	100 (3)	100 (3)	99 (8)	100 (3)	102 (3)	100 (5)	100 (4)
Dichlofluanid	91 (11)	93 (9)	**111** (10)	98 (13)	79 (7)	78 (8)	86 (10)	81 (9)	90 (15)
Dichlofluanid[b]	65 (5)	80 (3)	90 (3)	78 (14)	76 (2)	82 (8)	88 (2)	82 (9)	80 (12)
Dichlorobenzophenone	103 (12)	93 (5)	99 (7)	98 (9)	99 (6)	92 (5)	100 (5)	97 (6)	98 (8)
Dichlorvos	96 (13)	99 (3)	99 (3)	98 (7)	101 (10)	98 (2)	99 (2)	99 (6)	98 (7)
Dichlorvos[b]	103 (5)	100 (2)	99 (1)	101 (4)	97 (3)	95 (2)	96 (3)	96 (3)	98 (4)
Dieldrin	90 (7)	99 (6)	106 (6)	98 (9)	95 (12)	97 (4)	100 (6)	97 (8)	98 (8)
Endosulfan sulfate	**124** (**21**)	101 (5)	100 (8)	109 (**17**)	96 (**21**)	96 (11)	104 (9)	99 (14)	104 (**16**)
Folpet	—-	—-	—-	—-	—-	—-	86 (12)	—-	—-
Heptachlor epoxide	**116** (**23**)	102 (5)	100 (6)	101 (6)	—-	100 (6)	96 (9)	98 (7)	100 (6)
Hexachlorobenzene	100 (4)	95 (2)	96 (5)	97 (4)	94 (3)	92 (5)	92 (5)	93 (4)	95 (5)
Imazalil	—-	95 (8)	97 (7)	96 (7)	—-	98 (11)	94 (11)	96 (12)	96 (10)
Imazalil[b]	89 (7)	89 (2)	93 (2)	90 (5)	94 (3)	92 (1)	93 (3)	93 (2)	92 (4)
Imidacloprid[b]	109 (5)	100 (2)	98 (2)	102 (6)	102 (3)	97 (3)	95 (3)	98 (4)	100 (6)
Lindane	106 (5)	95 (3)	101 (4)	100 (6)	93 (7)	100 (5)	98 (5)	97 (6)	99 (6)
Methamidophos[b]	**131** (11)	101(3)	85 (3)	106 (**20**)	84 (3)	81 (2)	85 (3)	83 (5)	95 (**20**)
Penconazole	106 (6)	99 (5)	102 (6)	102 (6)	90 (10)	99 (5)	95 (5)	95 (7)	99 (8)
Penconazole[b]	95 (2)	94 (2)	93 (2)	94 (2)	96 (3)	96 (1)	97 (3)	96 (2)	95 (3)
Permethrins	**115** (8)	98 (8)	97 (7)	103 (10)	90 (8)	96 (10)	100 (7)	95 (9)	99 (10)
Propoxur	93 (9)	95 (5)	**114** (3)	101 (11)	87 (**18**)	98 (5)	98 (4)	95 (11)	98 (11)
Pymetrozine[b]	108 (12)	98 (2)	91 (2)	99 (11)	82 (9)	78 (2)	78 (9)	79 (6)	89 (14)
Thiabendazole	—-	99 (7)	97 (6)	98 (6)	—-	107 (13)	95 (9)	101 (12)	100 (10)
Thiabendazole[b]	108 (8)	98 (3)	97 (1)	101 (7)	88 (4)	92 (1)	90 (4)	90 (3)	95 (8)
Thiophanate-methyl[b]	102 (8)	98 (5)	95 (2)	98 (6)	106 (2)	97 (4)	97 (2)	100 (6)	99 (6)
Tolylfluanid	95 (**24**)	101 (9)	110 (12)	102 (**16**)	89 (5)	81 (12)	91 (9)	87 (9)	95 (**16**)
Tolylfluanid[b]	64 (9)	82 (3)	87 (4)	78 (14)	95 (4)	97 (6)	96 (4)	96 (4)	87 (14)

[a] Results in which the average recovery exceeded 110% and/or RSD was >15% are in bold; recoveries <80% are underlined.

[b] LC/MS/MS result.

values are needed only for monitoring unregistered pesticides, baby food in Europe (15), and some risk assessment applications. If matrix background is not the limiting source of noise, then LVI of the MeCN extracts can lower LOQ values further (6–8). Another option for GC analysis involves the use of analyte protectants, which can lower LOQs of relatively polar GC-amenable analytes, provide improved peak identification, and avoid the need for matrix-matched calibration standards (1, 12, 13).

Although we question the need for a solvent exchange and concentration step, many laboratories wish to continue injecting >1 mg sample equivalents in GC/MS and do not have LVI devices. If the extracts must be concentrated, then a convenient solvent exchange to a nonpolar organic solvent should also be performed. Our previous study (9) determined that toluene is the best exchange solvent in this circumstance for the following reasons: (*1*) it is miscible with MeCN, whereas iso-octane and hexane are not (and they float above MeCN, which complicates the solvent exchange); (*2*) it provides higher responses of methamidophos, acephate, and similar polar GC-amenable pesticides as compared to iso-octane and hexane; (*3*) it has a higher boiling point, thus volume changes due to solvent evaporation of the final extract are smaller, and it can serve as a keeper during evaporation steps; (*4*) the initial temperature in the GC oven program can be increased versus other solvents, thus saving re-equilibration times between injections in a sequence; (*5*) pesticides are very stable in toluene; and (*6*) the solvent vaporization expansion volume is relatively low, which permits >1 L injection volumes in typical splitless injection liners to potentially lower the LOQ further if needed. Drawbacks of toluene relate to: (*1*) its need for increased evaporation temperature in solvent exchanges, (*2*) high degree of solvent tailing on phenyl-based GC columns, (*3*) poor utility in LVI, and (*4*) incompatibility with polar analyte protectants.

In this study, we investigated the option of concentrating the final extracts into toluene to yield 5 g/mL equivalent sample concentration prior to GC/MS analysis. Table 4 shows the pesticide recoveries from this experiment, which can be compared to the GC/MS results in Table 3 for MeCN extracts. For the majority of pesticide analytes, the recovery and repeatability data from the method continued to remain exceptionally good (100 10% recoveries). Much of the credit can be attributed to the IS added to the extracts at the beginning of the method. During centrifugation of the final toluene extracts, 3 glass tubes among the 36 replicates broke, but the IS made those results indistinguishable from the others. However, problematic pesticides in GC/MS analysis remained problematic despite the 5-fold higher concentrations and use of a nonpolar solvent. The conditions of GC/MS were such that captan, dicofol, and folpet were still unable to be sensitively detected. As the results in Tables 3 and 4 indicate, no or only slight improvements in the LOQ were achieved for captan, chlorothalonil, and folpet. Only heptachlor epoxide at the 10 ng/g level in orange could be determined in the toluene extracts by GC/MS, but not in the MeCN extracts.

The peaks for chlorothalonil and folpet were missing or very small in the calibration standards for lettuce. We had expected that the storage stability of the base-sensitive pesticides would not be a problem in the toluene procedure, but the results in orange were satisfactory. Therefore, the less acidic conditions or another aspect of the lettuce must have been a contributing factor to degradation (but curiously occurred to a greater extent in the matrix-matched standards).

Coumaphos, lindane, and endosulfan sulfate, however, are considered to be stable, and their >110% recoveries in the lettuce extracts could not be easily explained. We hypothesize that potentially differing amounts of MeCN in the calibration standards from the spiked sample extracts in the injected toluene solutions may have been the cause of this effect. Big differences in the responses of these pesticides in MeCN or toluene were not observed previously (9), but the lettuce matrix may have been a compounding factor. Additional working standard solutions should be prepared in toluene in this case to minimize the possible effect of MeCN in the final extracts in toluene, and perhaps acid should be added to extracts to help ensure stability of base-sensitive pesticides.

In terms of sensitivity, the chromatographic peaks were generally 5 times larger (thus 5 times lower LOQ) for those analytes that give reliable GC/MS analysis (e.g., chlorpyrifos, diazinon, and DDE). No additional interferences were observed in the SIM mode versus the MeCN extracts, thus a 2–3 L toluene injection volume rather than 1 L for the same injection liner could have been used (in combination with a retention gap) to lower the LOQ even further. Therefore, the solvent exchange to toluene undeniably provided some benefits.

However, the cost in time and effort of using this option is significant. Foremost, the solvent exchange and concentration step increases the length of the entire procedure for a batch of extracts by >30 min. This more than doubles the time of the QuEChERS method, in which 10–20 preweighed homogenized samples can be prepared in 30–40 min by a single analyst. MeCN and toluene have rather high boiling points, so they take a long time to evaporate even at the relatively high temperature (50 C) and gas flow rate used in this procedure.

Less obvious costs relate to the larger sample size, tubes, and other materials needed to conduct the additional steps. The greater glassware needs alone undermine the elegant feature that an unbreakable, easily-washed FEP tube constitutes the only item needing to be cleaned in the streamlined QuEChERS method. The evaporation step also necessitates that an evaporation device be available, which increases capital expense and reduces the ability to perform sample preparation in the field or in a mobile minilaboratory. Moreover, each additional step in the method leads to greater potential for analyte losses and more variability in the results.

The most important consideration, however, relates to sample size. If a larger sample size is needed, then larger tubes, centrifuges, and rotors and more materials are required, which leads to a domino effect of greater expense, more space and labor needs, and lower sample throughput. Depending on

Table 4. Average % recoveries (%RSD) of fortified pesticides in lettuce and orange from the buffered QuEChERS method with GC/MS (SIM) analysis of final extracts concentrated and exchanged into toluene[a]

	Romaine lettuce				Orange				
Pesticide	10 ng/g (*n* = 6)	50 ng/g (*n* = 6)	250 ng/g (*n* = 6)	Overall (*n* = 18)	10 ng/g (*n* = 6)	50 ng/g (*n* = 6)	250 ng/g (*n* = 6)	Overall (*n* = 18)	Overall (*n* = 36)
Captan[b]	—-	—-	—-	—-	—-	—-	92 (14)	—-	—-
Chlordane	101 (1)	98 (1)	110 (2)	103 (5)	102 (6)	99 (2)	104 (6)	102 (5)	102 (5)
Chlorothalonil[b]	—-	—-	—-	—-	—-	42 (**20**)	87 (**23**)	65 (**41**)	—-
Chlorpyrifos	106 (6)	101 (1)	107 (2)	105 (4)	101 (5)	97 (2)	104 (6)	101 (5)	103 (5)
Chlorpyrifos-methyl	104 (2)	101 (2)	109 (5)	104 (4)	101 (4)	98 (3)	103 (5)	101 (4)	103 (5)
Coumaphos	107 (5)	**114** (4)	**126** (12)	**115** (10)	**116** (7)	99 (7)	**113** (11)	109 (10)	**112** (11)
DDE	97 (4)	95 (1)	106 (2)	99 (6)	98 (6)	96 (3)	104 (7)	99 (6)	99 (6)
Diazinon	100 (2)	102 (2)	100 (1)	100 (2)	99 (3)	98 (3)	104 (4)	100 (4)	100 (3)
Dichlorobenzophenone	99 (4)	97 (3)	104 (5)	100 (5)	95 (7)	97 (5)	108 (6)	100 (8)	100 (6)
Dichlorvos	91 (3)	98 (2)	88 (3)	93 (5)	102 (5)	97 (4)	98 (7)	99 (5)	96 (6)
Dieldrin	**111** (7)	101 (3)	**111** (2)	108 (6)	107 (6)	107 (4)	104 (6)	106 (5)	107 (6)
Endosulfan sulfate	**125** (10)	100 (10)	**139** (**22**)	**121** (**20**)	110 (10)	109 (11)	105 (**16**)	108 (11)	**115** (**18**)
Folpet[b]	—-	—-	—-	—-	—-	—-	94 (**34**)	—-	—-
Heptachlor epoxide	110 (5)	104 (4)	**111** (3)	108 (5)	101 (8)	96 (3)	105 (6)	101 (7)	104 (7)
Hexachlorobenzene	93 (12)	88 (1)	91 (1)	91 (7)	92 (3)	90 (2)	93 (4)	91 (3)	91 (5)
Lindane	108 (6)	**113** (6)	**126** (10)	**116** (10)	105 (6)	90 (3)	102 (7)	99 (9)	107 (12)
Permethrins	106 (5)	101 (5)	110 (3)	106 (6)	**111** (6)	106 (4)	**111** (5)	110 (5)	108 (6)
Propoxur	103 (13)	**118** (12)	**122** (13)	**114** (13)	**117** (4)	**111** (9)	96 (7)	108 (10)	**111** (12)

[a] *See* footnote a in Table 3.

[b] Detected in extracts but could not be quantified due to their losses in lettuce matrix-matched calibration standards.

the water content in the sample, a 1 mL/g sample addition of MeCN will not necessarily provide 1 mL of extract. Furthermore, dispersive SPE permits recovery of only 50% of the extract volume. If a variety of matrixes are to be analyzed, then the analyst can expect that no more than 1/3 of the original amount of MeCN added to the sample will be available after the cleanup step. Due to severe biases that occur when working with small extract and pipetting volumes, the method cannot be simply adjusted to smaller volumes without complicating precautions. Therefore, at least a 1 mL final toluene volume was required for a 5 g/mL extract, which needed a 5 mL MeCN extract after cleanup. This meant that 10 mL had to be used in dispersive SPE, which necessitated 500 mg PSA plus 1.5 g $MgSO_4$ in 15 mL centrifuge tubes for the 10 mL initial extract, a 15 g sample size, and a 15 mL MeCN extraction volume plus 6 g $MgSO_4$ + 1.5 g NaAc. That is one reason why we increased sample size from 10 to 15 g in this study in comparison to the original QuEChERS method (1). Fortunately, 15 g of a well-homogenized sample could still be extracted satisfactorily by shaking in the 50 mL FEP centrifuge tubes, but this could pose a problem for lower density foods, such as broccoli.

Another major drawback in the toluene exchange option involves extensive complications when employing matrix-matched calibration standards. For the MeCN extracts, only a 1 mL final extract volume is needed, thus a single 15 g blank sample can easily provide 5 calibration standards. For the same number of matrix-matched calibration standards in toluene, five 15 g blank samples are required. This entails 5 times the work and materials, and ca 2-fold the time and expense. Moreover, the use of toluene (unlike MeCN) does not allow the use of polar (insoluble in toluene) analyte protectants, a promising approach to eliminate the need for matrix-matched standards altogether (1, 12, 13).

Additional SPE Cleanup

An imperfection in the original QuEChERS method was that the dispersive-SPE procedure using PSA does not remove chlorophyll and sterols from extracts of leafy vegetables. The PSA retains fatty acids and other organic acids that are ubiquitous in foods quite well, but the green color from chlorophyll is only slightly reduced during the cleanup step. The chlorophyll does not interfere in GC/MS analysis of the pesticides, but it can build up in the injection port liner and increase the frequency of liner changes and column maintenance. As described previously (1, 16–18), the use of

GCB removes chlorophyll and sterols from the extracts in dispersive SPE, but it also strongly retains important pesticides with planar structures, such as thiabendazole, terbufos, and hexachlorobenzene. The option to conduct a solvent exchange to toluene conveniently provided another option in the method to employ cartridge-based SPE using GCB to potentially provide cleaner final extracts. The stronger elution solvent, toluene, would be expected to increase the recoveries of the planar pesticides, provided that adequate cleanup was still achieved. Although SPE cartridges are typically 2–3 times more expensive than purchasing the sorbents directly, the use of cartridges would save the time and trouble of preweighing sorbents in the laboratory for dispersive SPE, and 100% of the extract volume would be recovered during the cleanup step.

In a preliminary experiment, we determined that 3 or 6 mL toluene was needed to elute >70% of the hexachlorobenzene spiked onto a 250 or 500 mg GCB cartridge, respectively. In our QuEChERS trials using traditional SPE cleanup, a 500 mg PSA cartridge was placed on top of a 250 or 500 mg GCB cartridge that had already been preconditioned with 3 or 6 mL toluene (this preconditioning step was found to be necessary to reduce pesticide elution volumes and rinse the many contaminants from the GCB). A ca 1 cm layer of anhydrous $MgSO_4$ was placed on top of each sorbent bed in advance to remove trace amounts of water and improve cleanup (18). The SPE stack was further preconditioned with 6 mL MeCN, a 40 mL preweighed test tube was placed beneath the stack in the SPE manifold, and 10 mL Romaine lettuce extract from the buffered QuEChERS method was passed through the cartridges at ca 2 mL/min. Clogging of the frits with $MgSO_4$ was a problem in some instances, which slowed the flows and lengthened the time of the procedure considerably. MeCN (3 mL) was used to wash the unretained pesticides through the stack, and air was permitted to flow through the cartridges to collect as much eluant as possible. Then, the PSA cartridge was removed, 3 or 6 mL toluene was added to elute retained pesticides, and air was allowed to flow through the tube as before. GCB cartridges of 2 different sizes were obtained from two different vendors for comparison purposes, but both were found to behave similarly.

The dark green MeCN extracts became clear and colorless when passed through the SPE stack, but the first drop of toluene started to turn the solution olive green, which only became more intense as more toluene eluted from the GCB. These experiments indicated that the PSA cartridge alone removed 77% by weight of bulk coextractives, and before adding toluene, 95–96% of the original amount of coextractives were removed from the lettuce extracts. However, toluene elution lowered that amount to 86–87%. Thus, the GCB with toluene elution only provided an additional 10% removal of bulk coextractives versus PSA alone.

This approach could still be worthwhile depending on pesticide recoveries but, unfortunately, hexachlorobenzene and thiabendazole still gave <70% recoveries in the lettuce extracts. A more troubling factor was the reduced recoveries of other pesicides, particularly acephate (ca 50% recovery). Others using column-based SPE with PSA had reported lower recoveries for acephate and certain other relatively polar pesticides (16, 19), but dispersive SPE with PSA had not posed this problem. In additional experiments, we found that dispersive SPE with PSA provided 30% less effective cleanup of lettuce coextractives by weight than an equivalent sorbent/extract ratio in traditional SPE, but significantly higher recoveries of acephate and other important pesticides were achieved in the former approach. We were not willing to trade up to 40% lower recoveries for these important pesticides for ca 30% more cleanup that made no observable impact on reducing interferences or extending ruggedness in the analyses. Even though dispersive SPE does not provide more cleanup than column-based SPE, the higher pesticide recovery can be added to the long list of advantages over traditional SPE as previously reported (1).

Conclusions

The modifications designed for the QuEChERS method to improve the extraction and stability of problematic pesticides (pymetrozine, chlorothalonil, dicofol, dichlofluanid, captan, tolylfluanid, and folpet) were demonstrated to successfully meet the objectives of this study. We maintain that the sample preparation goals were met, but problems still remain in the routine GC/MS analysis of chlorothalonil, folpet, captan, and dicofol, which do not lend themselves to analysis in LC/MS/MS. Analyte protectants (12, 13) have not been shown to solve this continuing problem, but the monitoring of their degradation products can serve routine monitoring purposes in the meantime.

The buffered QuEChERS method was demonstrated to provide excellent and highly reproducible recoveries for a wide range of GC- and LC-amenable pesticides from matrixes with pH 2–7 (nor would nonfatty basic commodities pose any trouble). The capabilities of the modified method for fatty matrixes will be reported elsewhere (20), but the conclusion of that study indicated that octadecyl chemically bonded (C_{18}) sorbent must be used in addition to PSA in dispersive SPE to help remove lipids, and recoveries of the most nonpolar pesticides were reduced (presumably due to the incomplete dissolution of fats by MeCN during extraction).

On the basis of the validation results for Romaine lettuce and orange presented in this study, we have written a protocol and initiated an interlaboratory collaborative study of the buffered QuEChERS method using GC/MS and LC/MS/MS for analysis. The results from this extensive study will be reported in the future.

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